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DRUG DEVELOPMENT AGAINST VIRAL DISEASES

ANNUAL REPORT

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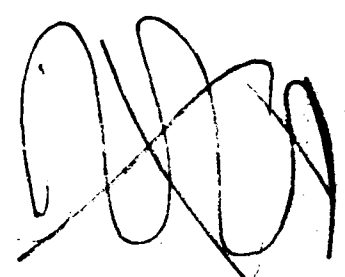
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19 ABSTRACT (Continue on reverse if necessary and identify by block number) One hundred fifty drugs have been tested for efficacy against CCHF (strain 10200). Sixteen percent of the drugs tested had VR (virus rating) scores greater than 1.5. Ten percent had VR scores greater than 1.8. An additional twelve drugs tested were found to be toxic at 50 mg/kg and in many cases at all levels tested, some as low as 0.25 mg/kg. The total number of drugs tested for toxicity in the CCHF model was one hundred sixty-two. The sensitivity of the CCHF test system was monitored by inclusion of the positive drug, ribavirin, AVS #1. The VR+ value was compared after 19 tests. The mean VR+ was 2.6 with standard deviation of 0.6. Tests were done with selected drugs to determine the effect of virus dose on VR scores using 50 and 5 LD50's of virus. The correlation between VR scores at the two dose levels was close with ineffective drugs. In contrast, with drugs giving a high VR score at 50 LD50's, the correlation at 5 LD50's was less close. However, there was little variation using the VR+ control drug, ribavirin. The pathogenesis of CCHF virus in infant mice was determined by titrating virus daily from virus-infected mice and					
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from ribavirin-treated mice using blood, liver, brain, spleen, and heart. The target organ of CCHF infection in this model was the liver. Virus titers were higher in the liver than in the blood from day 3 to day 7 (death of the mice). Virus appeared very late after infection in other tissues including the brain (day 7); heart (day 6); spleen (day 6). Ribavirin-treated mice showed a reduced viremia and lower virus titers in liver tissue.

One hundred seventy-two drugs were tested for efficacy in the LCMV model. Ten drugs were toxic giving a total of one hundred eighty-two drugs tested. Seven percent of the drugs tested had VR scores approximating or greater than 2.0. Histopathologic examination revealed that infected mice in our LCMV model died from a severe, multisystemic disease, with necrotizing inflammation of lymphoid tissues, parotid salivary glands, pancreas, splenic red pulp, liver, intestine and mesentery. Mice also had mild focal choriomeningitis. The majority of leukocytes, regardless of type, in all tissues examined were necrotic. No lesions were found in submaxillary or sublingual salivary gland, kidney, heart, eye, lacrimal gland, thyroid, trachea, or lung.

Nine drugs were tested in a rabies virus model of intramuscular infection. Two drugs showed promising results which should be explored further (VR's of 1.6 against 100 LD50's or virus).

Viremia levels were determined using sera from yellow fever virus infected and ribavirin-treated primates (squirrel monkeys). Statistically significant reductions in viremia levels were found in ribavirin-treated animals in the absence of significantly improved mortality. Alpha-beta interferon levels were undetectable or low in all infected animals.

FOREWORD

In conducting the research described in this report, the investigator adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW publication No. (NIH) 86-23, Revised 1985).



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TABLE OF CONTENTS

FOREWORD.....	1
INTRODUCTION	4
PRIMARY TESTING CONGO-CRIMEAN HEMORRHAGIC FEVER VIRUS (CCHF)	4
NUMBER OF DRUGS TESTED	4
DESCRIPTION OF THE MODEL AND ANALYSIS OF DATA.....	4
DRUG TESTING.....	4
ANALYSIS OF DATA.	4
PATHOGENESIS OF CCHF VIRUS IN INFANT MICE.	4
TEST REPRODUCIBILITY AND SENSITIVITY	5
VARIATION IN SENSITIVITY	5
REPRODUCIBILITY OF RESULTS.....	5
SUMMARY OF RESULTS OF DRUG TESTING.	5
DISTRIBUTION OF VR SCORES.....	5
EFFECT OF VIRUS DOSE ON VR SCORE	5
PRIMARY TESTING IN THE LCM MODEL.....	5
NUMBER OF DRUGS TESTED	5
DESCRIPTION OF THE LCMV MODEL	6
HISTOPATHOLOGY OF LCMV INFECTION.....	6
DISTRIBUTION OF LCMV VR SCORES	7
ANALYSIS OF REPRODUCIBILITY AND VARIATION.....	7
PRIMARY TESTING IN THE RABIES MODEL.....	8
NUMBER OF DRUGS TESTED	8
DESCRIPTION OF THE MODEL.....	8
SECONDARY TESTING IN THE YELLOW FEVER VIRUS MODEL	8
ANALYSIS OF DATA FROM YELLOW FEVER-RIBAVIRIN TESTS.	8
LIST OF TABLES	
TABLE 1. SUMMARY OF TEST RESULTS IN THE CCHF MODEL.....	9
TABLE 1A. TOXIC DRUGS IN THE CCHF MODEL.....	12
TABLE 2. SUMMARY OF TEST RESULTS IN THE LCM MODEL	20
TABLE 2A. TOXIC DRUGS IN THE LCMV MODEL.....	24
TABLE 3. SUMMARY OF RESULTS IN THE RABIES VIRUS MODEL	28
TABLE 4 VIREMIA IN YELLOW FEVER-RIBAVIRIN PRIMATE STUDY.....	29
LIST OF FIGURES	
FIGURE 1. CCHF VIRUS IN MOUSE TISSUES (BLOOD, LIVER, BRAIN)	13
FIGURE 2. CCHF VIRUS IN INFANT MOUSE TISSUES (LIVER AND HEART).....	13
FIGURE 3. CCHF VIRUS IN INFANT MOUSE TISSUES (LIVER AND SPLEEN)	14
FIGURE 4 EFFECT OF RIBAVIRIN ON VIREMIA IN INFANT MICE	15
FIGURE 5. EFFECT OF RIBAVIRIN ON CCHF VIRUS IN LIVER TISSUE.....	16
FIGURE 6. DISTRIBUTION OF VR+ SCORES IN THE CCHF MODEL	16
FIGURE 7. FREQUENCY DISTRIBUTION OF VR SCORES IN THE CCHF MODEL.....	17

**TABLE OF CONTENTS
(CONTINUED)**

FIGURE 8. VR SCORES AND PERCENTILE RANKING IN THE CCHF MODEL.....	18
FIGURE 9 CORRELATION BETWEEN CCHF VR SCORES AND VIRUS DOSE.....	19
FIGURE 10. FREQUENCY DISTRIBUTION OF LCM VR SCORES	25
FIGURE 11 LCM VR SCORES AND PERCENTILE RANKING	26
FIGURE 12. CORRELATION BETWEEN MULTIPLE TESTS IN THE LCM MODEL.....	27
FIGURE 13....DISTRIBUTION OF RABIES VIRUS VR SCORES	28
DISTRIBUTION LIST	30

INTRODUCTION

PRIMARY TESTING CONGO-CRIMEAN HEMORRHAGIC FEVER VIRUS (CCHF)

NUMBER OF DRUGS TESTED

ONE HUNDRED-FIFTY drugs have been tested for efficacy against CCHF (strain 10200). The results in virus ratings (VR) are given in Table 1. The details of the virus rating is given in a later section. An additional TWELVE drugs have been tested and were found to be toxic at 50 mg/kg and in many cases at all levels tested, some as low as 0.25 mg/kg. The total number of drugs tested is ONE HUNDRED AND SIXTY-TWO. Detailed data for each drug were submitted at periodic intervals throughout the year.

DESCRIPTION OF THE MODEL AND ANALYSIS OF DATA

DRUG TESTING

The CCHF model is as follows. Drugs have been tested for toxicity in infant mice (1-2 days old) at 50 mg/kg. Those which were not toxic were subjected to testing against CCHF, strain 10200, passage 11 in infant mouse brain tissue. Each drug was given to infant mice in a volume of 0.075 mls, i.p. Forty-five minutes later, virus at 50 LD₅₀'s was inoculated ip in a volume of 0.075 mls. Mock-treated mice were given tissue culture medium DMEM as control. Virus titrations were carried out at the same time. Mice were observed daily. All mice were housed in a modified class 3 facility using isolators. Mice were inspected for intercurrent murine infections throughout the year.

ANALYSIS OF DATA.

Geometric mean times to death were calculated for control mice (VC) and for drug-treated mice (VR). The geometric mean time to death (VC) is equal to the n th root (where n =the total number of animals) of the product of each day with mortality raised to the power of the number of animals dying on that day. In this calculation, survival is defined as 28 days. The geometric mean time to death (VR) for each drug is equal to the ratio of the geometric mean time to death for each drug divided by VC. As soon as a single positive drug was identified, that drug was incorporated into each test as a measure of test sensitivity. The drug chosen was ribavirin (AVS #1).

PATHOGENESIS OF CCHF VIRUS IN INFANT MICE.

Virus was titrated daily from virus infected mice and from ribavirin-treated mice using blood, liver, brain, spleen, and heart in SW-13 cells. Titers were determined by fluorescent assay. Virus infection was associated with multiplication in the liver. Virus titers were higher in the liver than in the blood (Figure 1) from day 3 to day 7 (death of the mice). Virus appeared very late after infection in other tissues including the brain (day 7, Figure 1); heart (day 6, Figure 2); spleen (day 6, Figure 3). Ribavirin-treated mice showed a reduced viremia (Figure 4) and lower virus titers in liver tissue (Figure 5).

TEST REPRODUCIBILITY AND SENSITIVITY

VARIATION IN SENSITIVITY

The sensitivity of the test system was monitored by inclusion of the positive drug (AVS #1). The VR+ value was compared after 19 tests. The mean VR+ was 2.6 with a minimum of 1.6 and a maximum of 3.5 (range of 1.9) giving a standard deviation of 0.6. A histogram giving the distribution of VR+ scores is given in Figure 6. As can be seen from the histogram, most observations (12/19, 63%) were between 1.6 and 2.0.

REPRODUCIBILITY OF RESULTS.

One drug AVS #206 was tested four different times. The VR scores ranged from 1.9, 2.6, 3.0, 3.0, showing a range similar to that of AVS #1, the positive control. The mean VR was 2.6 with a standard deviation of 0.5.

SUMMARY OF RESULTS OF DRUG TESTING.

DISTRIBUTION OF VR SCORES.

The result of testing of 147 drugs is given in Table 1. The distribution of VR scores is shown in a histogram in Figure 7. The vast majority of VR scores falls between 0.9 and 1.2 (67%). Only 16% of the drugs tested had VR scores greater than 1.5. Only 10% had scores greater than 1.8. In Table 1, drugs giving VR scores greater than 1.5 are shown in bold type. The percentile ranking for each VR score is given in Figure 8.

EFFECT OF VIRUS DOSE ON VR SCORE

Tests were done with selected drugs to determine the effect of virus dose on VR scores using 50 and 5 LD50's of virus. The correlation between VR scores at the two dose levels was close with ineffective drugs. For example, using a drug which had a VR of 1.0 against 50 LD50's, the VR's against 5 LD50's ranged from 0.9 to 1.4. In contrast, with drugs giving higher VR scores at 50 LD50's, the correlation at 5 LD50's was less close. For example, at 50 LD50's of virus, VR scores of 1.6 and 1.8 became scores of 0.9 and 1.4 respectively at 5 LD50's of virus. A correlation scattergram is shown in Figure 9, complete with a regression line. However, variation with virus dose using the positive control drug, AVS#1, in these same experiments, was far less marked than the variation seen with other test drugs. For example, VR+ scores against 50 LD50's of virus were 2.4, 2.8, 2.6, 2.3 and against 5 LD50's of virus were 2.6, 2.3, 2.7, 2.1 respectively. Thus, minor variations in the test virus dose cannot explain all the variation observed in this model system. Fresh drugs doses are prepared for all tests, so that variable is controlled as far as possible.

PRIMARY TESTING IN THE LCM MODEL

NUMBER OF DRUGS TESTED

ONE HUNDRED AND SEVENTY-TWO drugs have been tested in the LCM model. VR scores have been sent to the contract officer as they became available to us. The detailed data are not, therefore, included in this report. However, a summary of our results is presented in Table 2.

DESCRIPTION OF THE LCMV MODEL

Adult mice are inoculated with 50 mg/kg of drug i.p. in a volume of 0.4 mls. Forty-five minutes later, mice are inoculated with 50 LD50's of LCM virus (LCMV) i.p. The virus strain is propagated by intracerebral passage in inbred C3H mice. Random bred CF-1 mice from Charles River are used for drug tests. The identity of the virus strain has been monitored by examination of infected mouse tissue by immunofluorescence. Considerable effort was expended during the year on restoring the high peripheral virulence of this strain of LCMV. This was accomplished by serial passage through murine neuroblastoma cells (clone N18), followed by intracerebral passage of virus. At this point, the virus stock titers 5.0 to 5.4 log LD50's by intraperitoneal inoculation of random bred mice.

HISTOPATHOLOGY OF LCMV INFECTION

Adult random-bred mice inoculated with 50 LD50's of LCMV were examined on days 10 and 11 post inoculation.

In most tissues, there was diffuse hyperemia and congestion of vessels, with margination and migration of leukocytes through venous walls. Most leukocytes in tissues and vessel lumina were remarkably pyknotic. All lymphoid tissues (thymus, cervical lymph nodes, mesenteric, lymph nodes, spleen, Peyer's patches) had marked depletion of lymphocytes, with necrosis of most remaining lymphocytes, with early lymphoid regeneration in some foci. Parotid salivary glands (but not submaxillary or sublingual) had severe necrotizing sialoadenitis, with acinar necrosis and edema of interstitial, interlobular and interlobar connective tissue. Pancreas had mild acinar epithelial necrosis with edema. Splenic red pulp had myeloid hyperplasia, with necrosis of mature myeloid cells. Liver had moderate generalized peripherolobular hepatocellular hydropic change with necrosis of many individual hepatocytes, portal hepatitis and sinusoidal hypercellularity due to Kupffer cell hypertrophy, leukocytic infiltration and extramedullary myelopoiesis. Intestine had mild but general epithelial degeneration, with excessive exfoliation into bowel and crypt lumina. Leukocytes in lamina propria were pyknotic. There was mild, diffuse mucosal hyperplasia and lymphoangiectasia. Brains had mild multifocal leptomeningitis, perivascularitis of Virchow-Robin spaces, ependymitis and choroiditis. Pyknosis of a few parenchymal cells was present adjacent to these areas. Mesenteric tissue was inflamed with edema, leukocytic infiltration and fat necrosis. Kidneys, heart, eye, Harderian gland, thyroid, trachea and lung were unaffected.

CONCLUSION AND SUMMARY OF LCMV HISTOPATHOLOGY

Mice were suffering from severe, multisystemic disease, with necrotizing inflammation of lymphoid tissues, parotid salivary glands, pancreas, splenic red pulp, liver, intestine and mesentery. They also had mild focal choriomeningitis. The majority of leukocytes, regardless of type, in all tissues examined were undergoing necrosis. No lesions were found in submaxillary or sublingual salivary gland, kidney, heart, eye, lacrimal gland, thyroid, trachea, or lung.

Lesions were consistent with visceral LCM disease (Lehmann-Grube, F. 1982. The Mouse in Biomedical Research. Vol II. Diseases. Edited by H. L. Foster, J.D. Small and J.G. Fox. New York; Academic Press.

DISTRIBUTION OF LCMV VR SCORES

A histogram giving the frequency distribution of VR scores is shown in Figure 10. The mean VR score and the mode of this distribution is 1.0 and 1.077 respectively. The relationship between VR score and percentile ranking is given in Figure 11. VR scores of 1.5 or higher are in the 95% of all drugs tested. In Table 2, drugs with scores of 1.5 or higher are shown in bold type. Statistical significance is not implied. (See following section.)

ANALYSIS OF REPRODUCIBILITY AND VARIATION.

Variation in the test system was low in some cases and higher in others. For example, AVS # 646 was tested twice. The results were 1.1 and 1.4. AVS # 206 was tested three times: VR 1.8, 1.5, and 0.9. (The 0.9 value for AVS 206 was obtained with a test dose of 250 LD50's., illustrating the importance of virus dose in determining VR values; the remaining VR scores were obtained against 50 LD50's of virus.) AVS #1 was tested five times with the following VR's: 1.9, 1.0, 1.5, 1.3, 1.1. The standard deviation of AVS#1 is 0.4. In the CCHF system, the standard deviation of the VR+ AVS #1 was 0.6. Thus, there was less variation in the LCM model, but there was also less consistent positive effect on survival. AVS #52 was tested twice: VR 1.0 and 1.2. Drug #253 was tested twice: VR 2.6 and 1.5. AVS #332 was tested twice with the following results: VR 1.1 and 1.0. AVS #345 was tested twice: VR 1.0 and 1.0. Drug #646 was also tested twice: VR 1.4 and 1.1. A scattergram of the variation between multiple tests is shown in Figure 12. These data show that a score of 2.0 in the LCMV model could reliably be considered to be representative of a reproducible positive effect. Because of the variation in test scores, VR values less than 2.0 are of less certain positive effect and might require additional testing for confirmation.

PRIMARY TESTING IN THE RABIES MODEL

NUMBER OF DRUGS TESTED

NINE drugs were tested for efficacy in preventing death from intramuscular rabies virus infection. The results of testing are shown in Table 3. Promising results were obtained with two drugs which gave VR scores of 1.6

DESCRIPTION OF THE MODEL

Rabies virus (100 i.m. LD50's, strain 1820B) was inoculated intramuscularly (0.03 mls) in adult random bred mice (Charles River, CF-1 strain). Drugs were given in a total dose of 50 mg/kg at the site of inoculation (0.2 mls) and i.p. (0.4 mls) Two types of experiments were performed. In single dose experiments, one dose was given forty-five minutes before virus. In multiple dose experiments, drug was given one day before virus, the day of inoculation, and the following two days for a total of four drug doses. Each drug dose was 50 mg/kg. The V(C) in both experiments was 5.0. Detailed data were submitted during the past contracting year.

SECONDARY TESTING IN THE YELLOW FEVER VIRUS MODEL

ANALYSIS OF DATA FROM YELLOW FEVER-RIBAVIRIN TESTS.

Viremia levels were determined from monkeys given yellow fever virus alone and from those given virus and ribavirin. In experiments completed in the previous contract period, prolonged survival, but no statistically significant reduction in mortality was seen in drug-treated animals. In the present contract year, we have determined that viremia levels were significantly reduced in those animals which were treated with ribavirin. These data are shown in Table 4. Alpha-beta interferon levels were low in both groups of animals with no differences observed between the two groups. One untreated monkey (87) had detectable levels (1:150) beginning on the 5th day after infection and lasting until the seventh day. Interestingly, this was a surviving monkey. However, among ribavirin treated animals, only one (86) of three surviving animals developed detectable levels of interferon (day 6-8). Clearly, interferon development was late, suggesting that interferon-stimulating drugs or other immunomodulating drugs might be of benefit in preventing death in ribavirin-treated animals.

TABLE 1. SUMMARY OF TEST RESULTS IN THE CCHF MODEL

AVS NUMBER	VR SCORE	VR+
33	1.1	2.7
71	0.9	2.7
78	0.9	2.7
206	1.9	1.8
206	2.6	3.0
206	3.0	3.0
206	3.0	3.1
1915	0.8	2.7
1968	1.5	2.7
1970	0.8	2.7
1977	1.1	2.7
1986	1.0	1.8
1989	1.0	2.7
2023	0.9	2.7
2137	1.8	2.7
2277	0.7	1.8
2279	0.7	1.8
2282	0.7	1.8
2290	0.9	1.8
2328	0.6	1.8
2335	0.8	1.8
2362	1.1	2.8
2381	1.4	2.5
2384	1.8	2.5
2388	1.0	2.5
2389	1.2	2.5
2390	1.1	2.8
2394	1.8	2.5
2400	1.0	2.5
2401	1.3	2.5
2543	1.1	2.8
2565	1.1	2.8
2685	1.1	2.8
2686	1.1	2.6
2687	1.0	2.8
2694	1.0	2.8
2695	1.0	2.6
2696	1.9	2.6
2697	1.2	2.6

TABLE 1 (CONTINUED)
SUMMARY OF TEST RESULTS IN THE CCHF MODEL

AVS NUMBER	VR SCORE	VR+
2698	1.1	2.8
2699	1.0	2.8
2700	1.2	2.8
2701	1.1	2.6
2702	1.0	2.3
2703	1.0	3.5
2704	1.0	2.3
2705	1.2	2.3
2707	1.6	2.3
2710	1.1	2.3
2711	1.0	3.5
2714	1.0	3.5
2716	1.1	3.5
2717	1.0	3.5
2718	0.9	3.5
2739	1.1	3.5
2740	1.1	3.5
2741	1.0	2.6
2742	1.1	2.6
2744	1.0	3.5
2745	1.0	3.5
2770	1.1	3.5
2771	1.1	3.5
2772	1.2	3.5
2773	0.7	2.3
2774	0.8	2.3
2775	0.9	2.3
2786	0.9	3.3
2787	0.9	3.3
2788	1.1	3.3
2789	1.0	1.8
2790	0.9	3.3
2869	1.0	3.3
2870	0.9	3.3
2871	1.9	3.3
2872	2.2	3.3
2873	1.3	3.3
2879	1.0	3.3
2889	0.9	1.8

TABLE 1 (CONTINUED)
SUMMARY OF TEST RESULTS IN THE CCHF MODEL

AVS NUMBER	VR SCORE	VR+
2890	0.9	1.8
2891	0.9	1.8
2895	1.9	1.6
2896	1.5	1.6
2906	1.7	1.6
2907	1.9	1.6
2908	1.5	1.6
2909	1.2	1.6
2910	1.0	1.6
2911	1.7	1.6
2912	1.4	1.6
2913	1.1	1.6
2914	2.3	1.6
2915	1.0	1.6
2916	1.0	1.6
2917	1.2	1.6
2918	2.1	1.6
2919	3.4	1.6
2930	2.5	3.1
2931	1.4	3.1
2932	1.2	3.1
2934	1.5	3.1
2936	1.0	3.1
2944	1.0	3.1
2947	1.1	3.1
2948	1.1	3.1
2956	1.0	3.1
2957	1.0	3.1
2958	1.4	3.1
2959	1.1	3.1
2960	1.0	3.1
2962	1.1	3.1
2963	1.0	3.1
2964	1.0	3.1
2966	1.1	3.2
2967	1.1	3.2
2970	1.0	3.2
2971	1.1	3.2
2972	1.0	3.2
2973	1.1	3.2

TABLE 1 (CONTINUED)
SUMMARY OF TEST RESULTS IN THE CCHF MODEL

AVS NUMBER	VR SCORE	VR+
2974	1.0	3.2
2975	1.1	3.2
2979	1.1	3.2
2981	1.0	3.2
2986	1.1	3.2
2989	1.1	3.2
2990	1.0	3.4
2991	1.0	3.2
2993	1.1	3.2
2994	1.0	3.2
2995	1.0	3.2
2996	1.0	3.4
3035	1.1	3.4
3036	1.0	3.4
3037	1.1	3.4
3039	1.1	3.2
3040	1.0	3.4
3041	1.1	3.2
3042	1.0	3.4
3043	0.9	3.4
3521	1.0	3.4
3522	1.1	3.4
3523	1.0	3.4
3524	1.0	3.4
3525	1.0	3.2
3526	1.2	3.2
3527	1.0	3.4
3528	1.0	3.4
3529	1.7	3.4
3593	1.1	3.4

TABLE 1A. TOXIC DRUGS IN THE CCHF MODEL

1160	2395
1990	2397
2139	2946
2140	2949
2382	2961
2383	2980

FIGURE 1. CCHF VIRUS IN MOUSE TISSUES (BLOOD, LIVER, BRAIN)

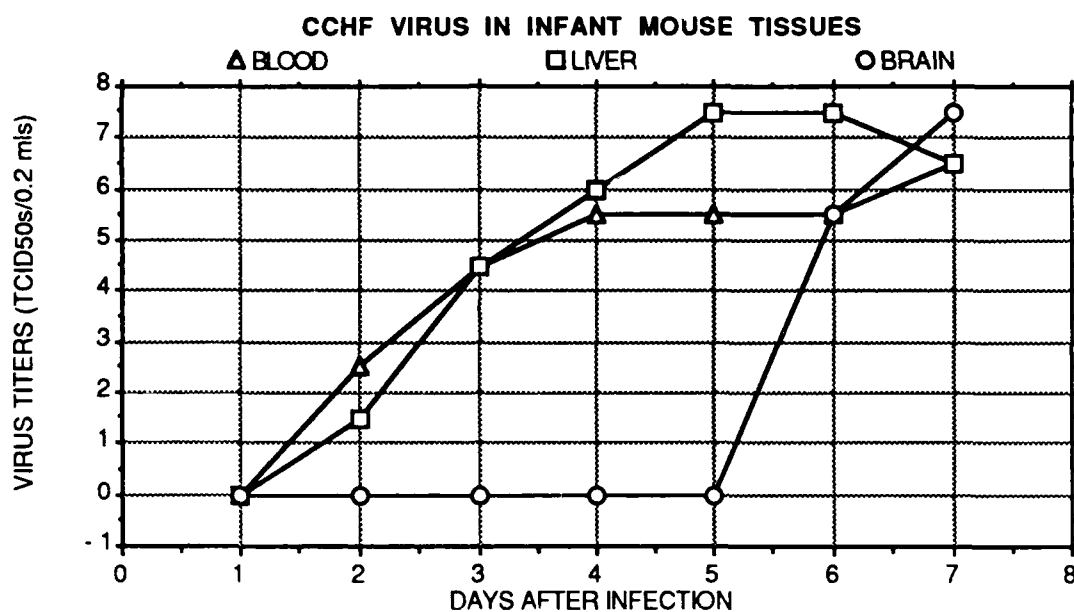


FIGURE 2. CCHF VIRUS IN INFANT MOUSE TISSUES (LIVER AND HEART)

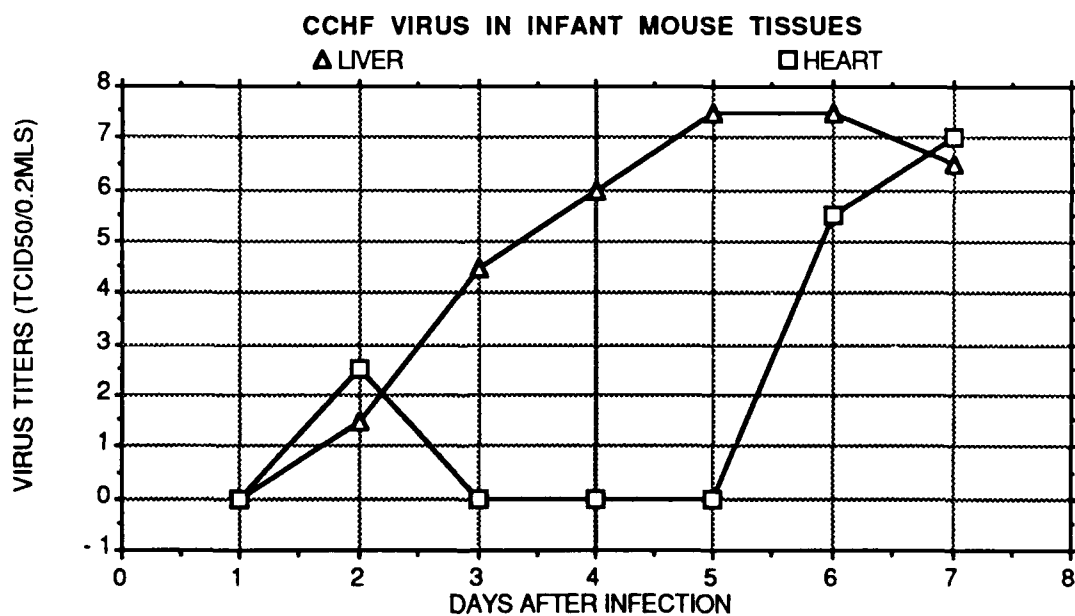


FIGURE 3. CCHF VIRUS IN INFANT MOUSE TISSUES (LIVER AND SPLEEN)

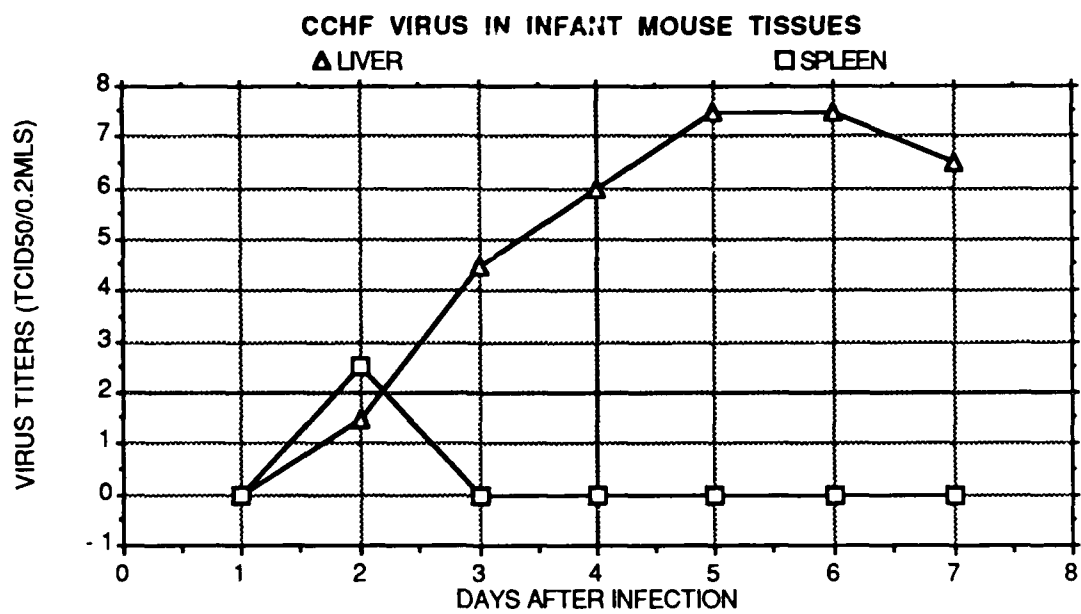


FIGURE 4 EFFECT OF RIBAVIRIN ON VIREMIA IN INFANT MICE

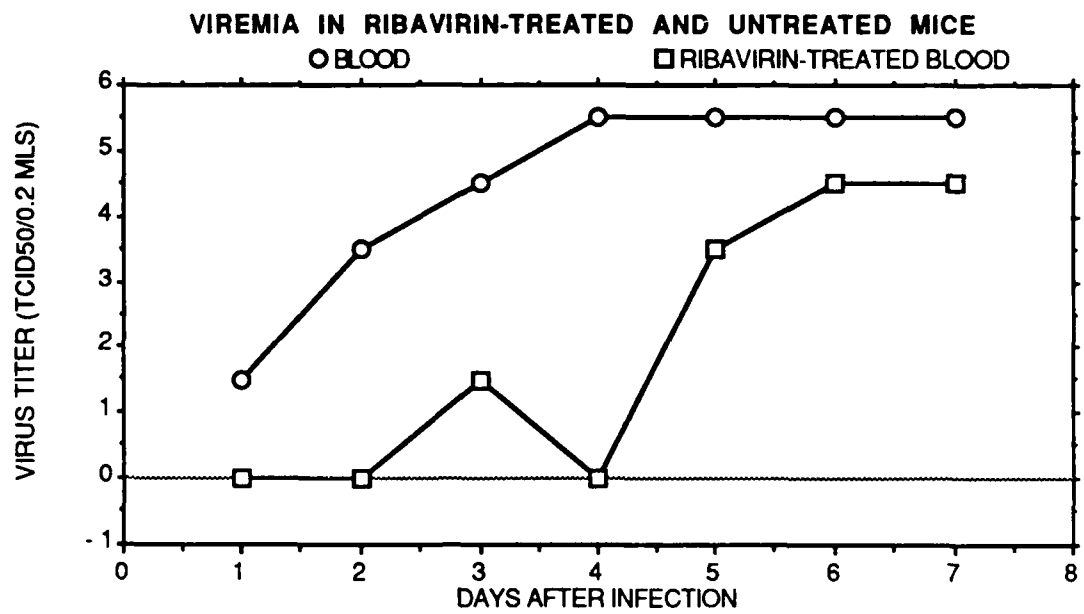


FIGURE 5. EFFECT OF RIBAVIRIN ON CCHF VIRUS IN LIVER TISSUE.

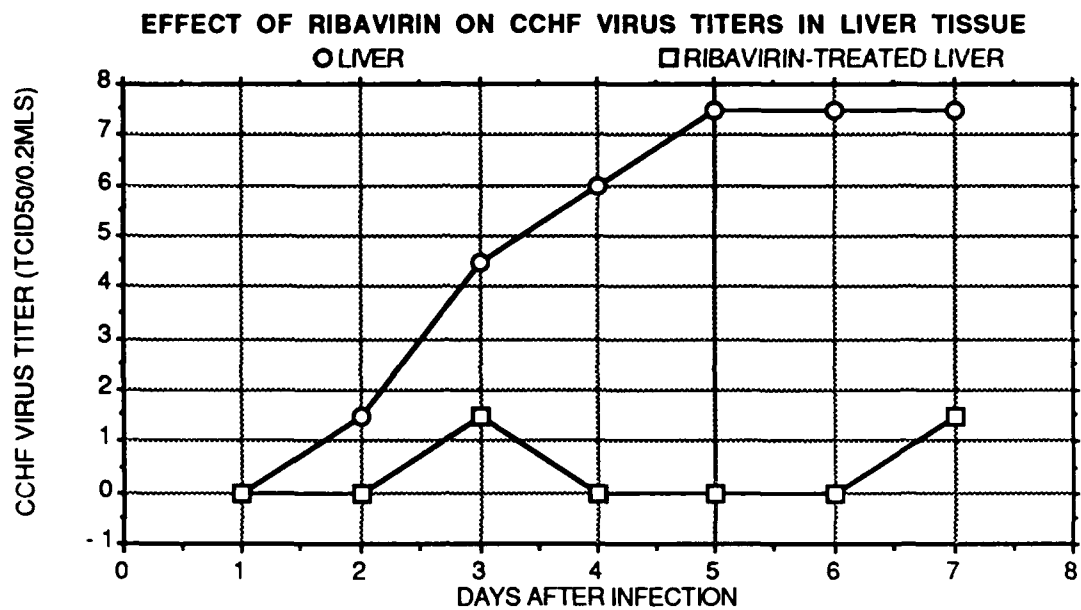


FIGURE 6. DISTRIBUTION OF VR+ SCORES IN THE CCHF MODEL

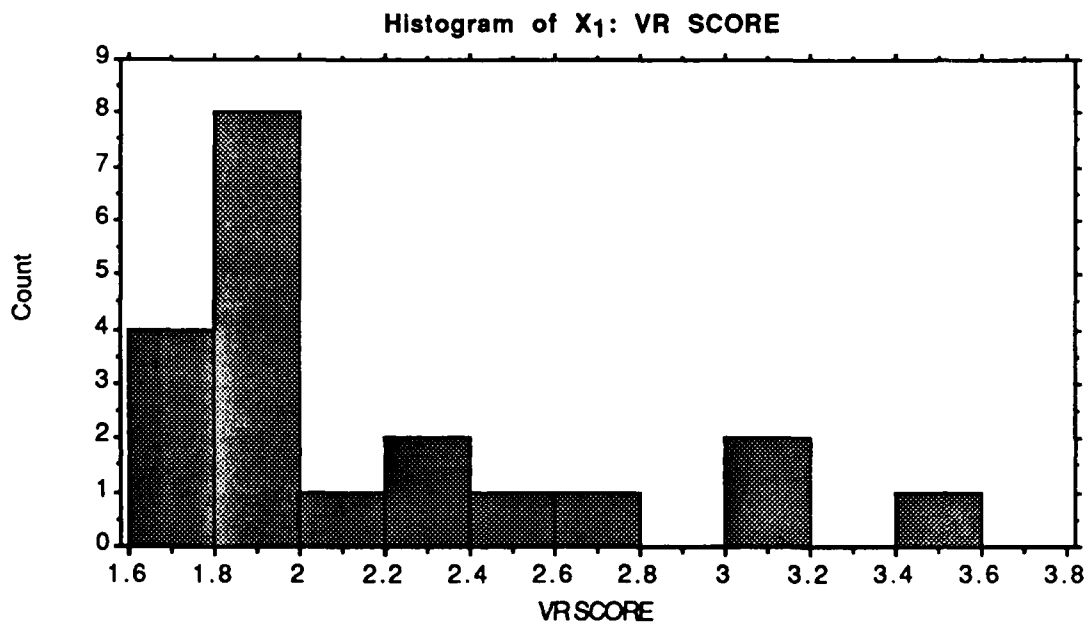


FIGURE 7. FREQUENCY DISTRIBUTION OF VR SCORES IN THE CCHF MODEL

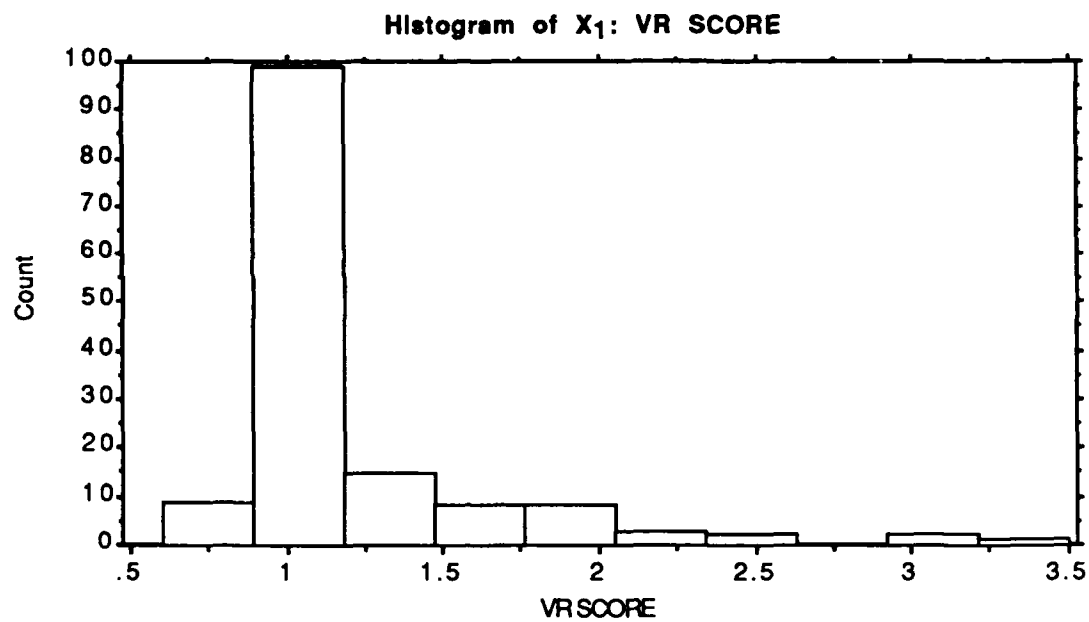
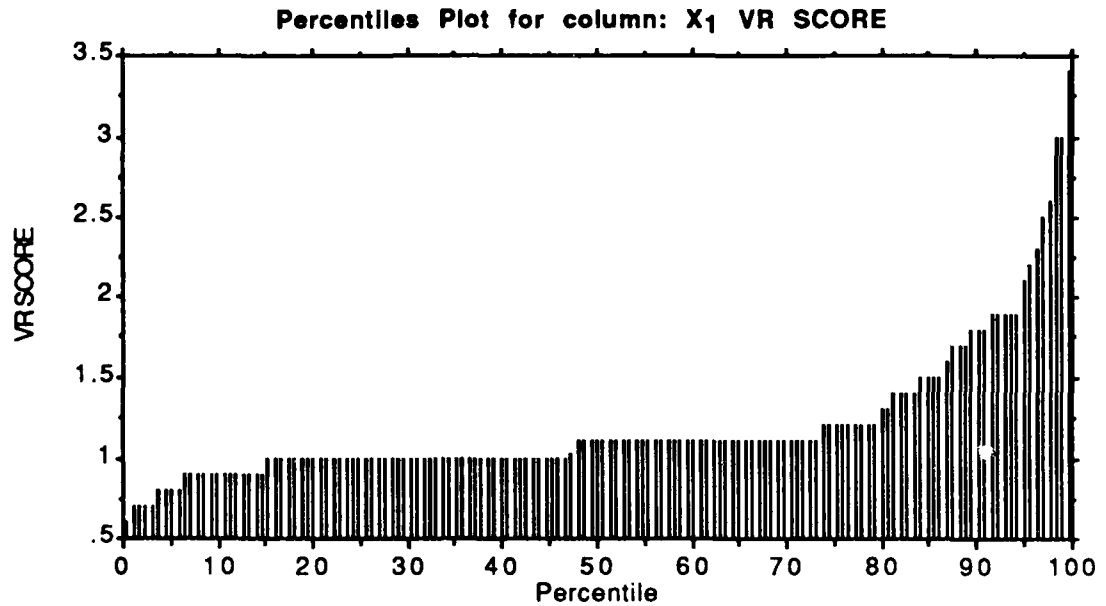


FIGURE 8. VR SCORES AND PERCENTILE RANKING IN THE CCHF MODEL



X₁: VR SCORE

Bar:	From: (\geq)	To: ($<$)	Count:	Percent:
1	1.6	1.8	4	2.721
2	1.8	2	8	5.442
3	2	2.2	1	.68
4	2.2	2.4	2	1.361
5	2.4	2.6	1	.68
6	2.6	2.8	1	.68
7	2.8	3	0	0
8	3	3.2	2	1.361
9	3.2	3.4	0	0
10	3.4	3.6	1	.68

-Mode

FIGURE 9 CORRELATION BETWEEN CCHF VR SCORES AND VIRUS DOSE

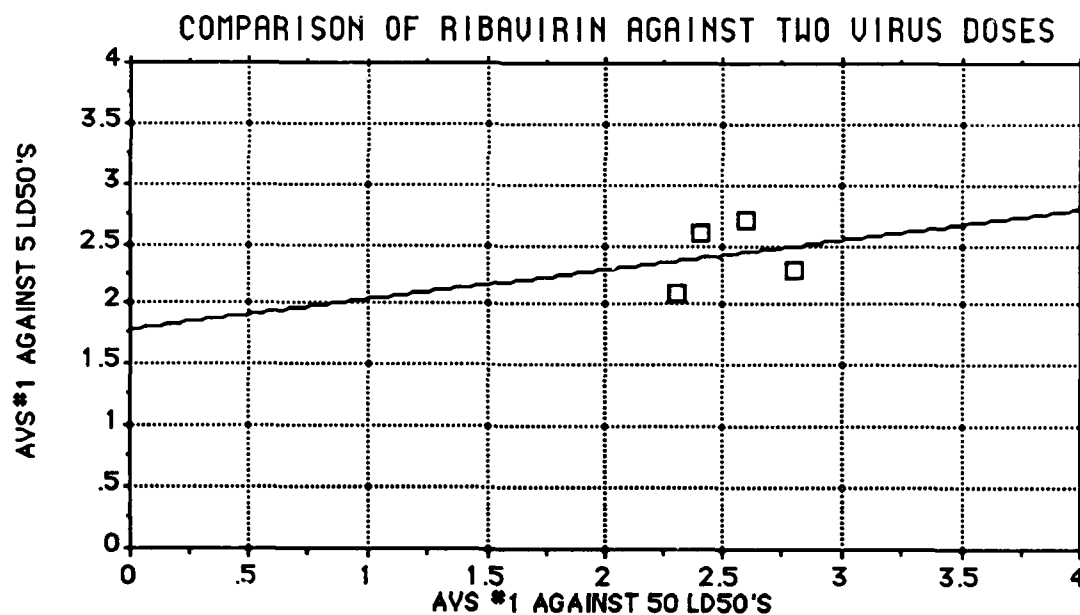
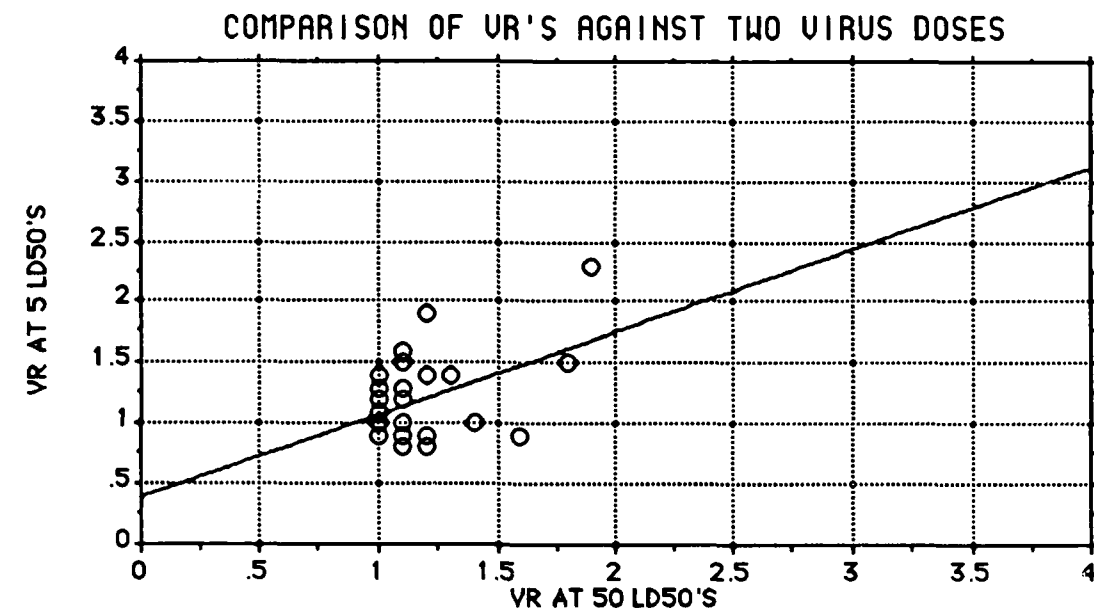


TABLE 2. SUMMARY OF TEST RESULTS IN THE LCM MODEL

AVS NUMBER	VR SCORE
1	1.9
1	1.0
1	1.5
1	1.3
1	1.1
33	1.0
52	1.0
52	1.2
64	1.6
79	1.1
84	1.1
87	1.3
94	1.2
95	0.8
95	1.0
111	1.0
136	1.0
181	1.2
206	1.8
206	1.5
206	0.9
215	1.1
253	2.6
253	1.5
257	1.5
332	1.1
332	1.0
345	1.0
345	1.0
360	1.0
361	1.2
593	1.0
646	1.1
646	1.4
701	0.7
1089	1.9
1160	2.6
1846	1.5

TABLE 2 (CONTINUED)
SUMMARY OF TEST RESULTS IN THE LCM MODEL

AVS NUMBER	VR SCORE
1915	1.2
1968	1.2
1970	0.8
1976	1.4
1977	1.1
1978	1.1
1983	1.6
1984	1.0
1985	0.9
1986	1.1
1987	1.2
1989	0.9
2137	1.0
2138	1.1
2388	0.8
2389	1.0
2397	1.2
2484	0.9
2507	1.0
2538	1.0
2540	1.0
2541	1.0
2542	0.9
2543	1.3
2567	1.0
2568	1.0
2569	1.0
2686	0.9
2687	0.9
2694	0.9
2695	1.0
2696	1.1
2697	0.9
2698	0.9
2699	1.1
2700	1.0
2741	1.3
2742	1.4

TABLE 2 (CONTINUED)
SUMMARY OF TEST RESULTS IN THE LCM MODEL

AVS NUMBER	VR SCORE
2786	1.4
2787	2.2
2788	1.2
2790	1.2
2869	1.0
2870	1.0
2872	0.9
2873	1.0
2874	0.9
2875	0.9
2876	0.9
2877	0.9
2878	1.2
2879	0.8
2883	0.9
2884	0.9
2885	1.0
2886	0.9
2888	1.1
2889	1.5
2890	1.1
2891	0.8
2893	1.0
2894	0.9
2895	1.1
2896	1.0
2906	1.4
2907	1.0
2908	0.9
2909	0.9
2910	0.9
2911	0.9
2912	0.9
2913	0.9
2914	0.9
2915	0.9
2916	0.9
2917	0.8

TABLE 2 (CONTINUED)
SUMMARY OF TEST RESULTS IN THE LCM MODEL

AVS NUMBER	VR SCORE
2918	1.4
2919	1.3
2926	0.7
2927	1.3
2929	1.2
2932	1.1
2936	1.3
2944	1.1
2946	0.9
2956	0.9
2957	1.8
2958	0.8
2960	1.1
2961	1.3
2964	1.0
2969	1.0
2970	1.0
2971	1.0
2972	1.0
2973	1.5
2974	1.2
2975	1.0
2978	1.0
2980	1.0
2981	1.5
2982	1.0
2983	1.0
2984	1.0
2985	1.0
2986	1.2
2987	1.1
2988	1.0
2989	0.9
2990	1.0
2991	1.2
2992	1.0
2993	1.6
2994	1.0

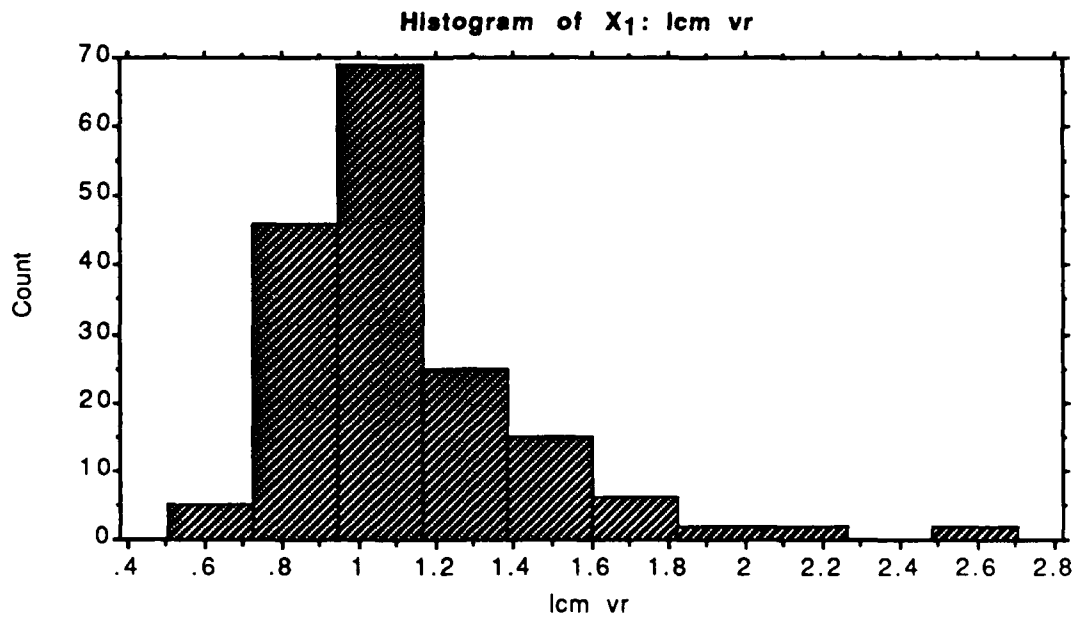
TABLE 2. (CONTINUED)
SUMMARY OF TEST RESULTS IN THE LCM MODEL

AVS NUMBER	VR SCORE
2995	1.1
2996	1.1
3035	0.5
3037	0.8
3039	1.3
3040	0.9
3041	0.8
3042	0.9
3043	0.7
3520	0.9
3521	0.8
3522	1.1
3523	1.0
3524	1.3
3525	2.1
3526	0.9
3527	0.7
3528	1.5
3593	0.8
3909	1.8

TABLE 2A. TOXIC DRUGS IN THE LCMV MODEL

70	1841
200	1901
272	2139
347	2140
1831	2949

FIGURE 10. FREQUENCY DISTRIBUTION OF LCM VR SCORES



X_1 : lcm vr

Bar:	From: (\geq)	To: ($<$)	Count:	Percent:
1	.5	.72	5	2.907
2	.72	.94	46	26.744
3	.94	1.16	69	40.116
4	1.16	1.38	25	14.535
5	1.38	1.6	15	8.721
6	1.6	1.82	6	3.488
7	1.82	2.04	2	1.163
8	2.04	2.26	2	1.163
9	2.26	2.48	0	0
10	2.48	2.7	2	1.163

-Mode

FIGURE 11 LCM VR SCORES AND PERCENTILE RANKING

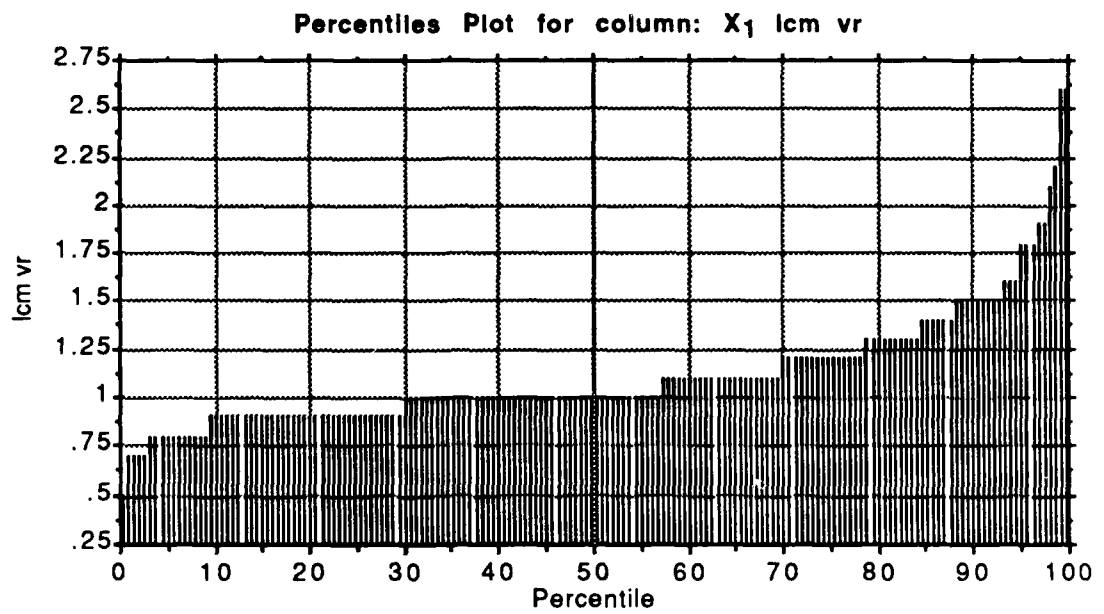
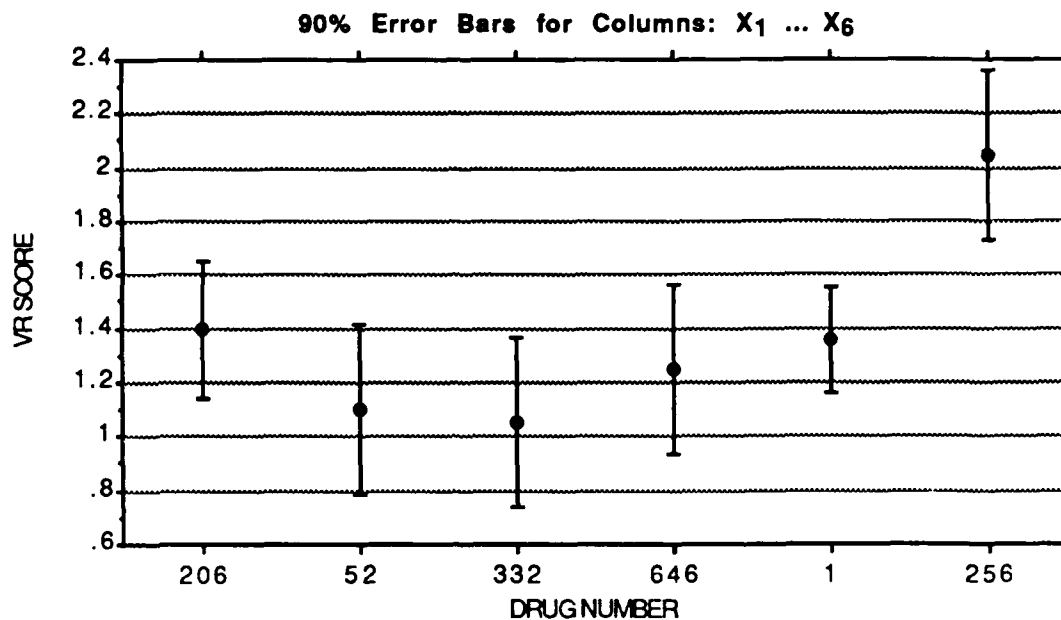


FIGURE 12. CORRELATION BETWEEN MULTIPLE TESTS IN THE LCM MODEL

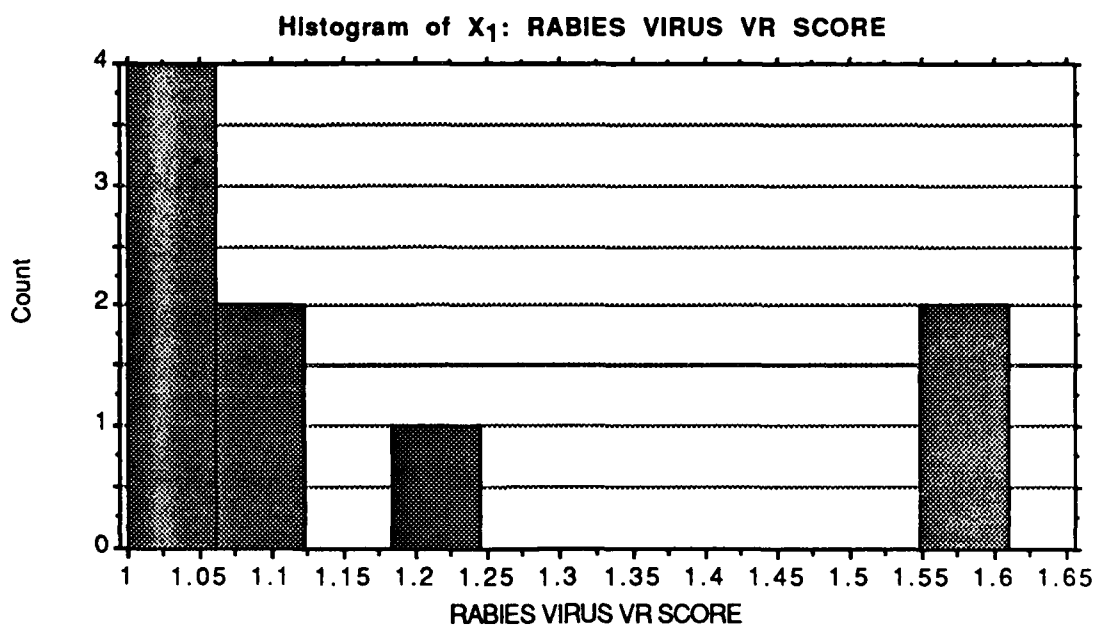


*The drug number 256 in this graph should be 253.

TABLE 3. SUMMARY OF RESULTS IN THE RABIES VIRUS MODEL

AVS NUMBER	VR RATING	
	SINGLE DOSE	MULTIPLE DOSE
1	1.0	1.0
1005	1.0	1.1
1028	1.0	1.1
1059	1.1	1.1
1159	1.0	1.6
1160	1.0	1.0
1207	1.0	1.6
1245	1.0	1.0
2906	1.0	1.0

FIGURE 13....DISTRIBUTION OF RABIES VIRUS VR SCORES



*FIGURE 13 GIVES THE DISTRIBUTION OF VR SCORES FOR MULTIPLE DOSE TESTS ONLY

VIREMIA IN YELLOW FEVER-RIBAVIRIN PRIMATE STUDY

		VIREMIA TITERS (LOG ₁₀ PFU'S/ML)									
TREATMENT		DAYS AFTER INFECTION								MORTALITY	
		1	2	3	4	5	6	7	8		
VIRUS ALONE											
87		0	3.7	5.4	3.4	0	0	0	0		
88		1.4	3.4	5.4	4.7	2.0	DIED				
89		1.2	3.4	5.4	5.4	2.3	1.8	0	DIED	5/6 83%	
90		1.7	3.7	6.5	DIED						
91		1.2	3.4	5.7	5.4	DIED					
92		4.5	3.5	7.4	DIED						
		GEOMETRIC MEAN TITER									
		1.7	3.5	6.0	4.7	1.4	0.9	0	0		
VIRUS AND DRUG											
86		0	1.9	3.4	5.4	3.2	0	0	0		
93		1.2	3.4	5.4	5.4	DIED					
94		0.7	2.0	3.4	5.5	3.7	4.9	DIED		2/5 40%	
97		0	0	0	0	0	0	0	0		
99		1.8	1.8	3.4	3.4	3.4	3.4	0	0		
		GEOMETRIC MEAN TITER									
		0.7	1.8	3.1	3.9	3.1	1.2	0	0		
DRUG INDUCED % DECREASE IN GEOMETRIC MEAN VIREMIA TITER											
		90	98	99.9	84	-	-	-	-		

BY SPEARMAN RANK CORRELATION COEFFICIENT, THESE DIFFERENCES IN VIREMIA LEVELS ARE SIGNIFICANT AT A PROBABILITY LEVEL BETWEEN 0.05 AND 0.01.

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